Primordial germ cell migration and the Wnt signaling pathway

Andrea V. *Cantú*, Diana J. *Laird*[1]

Department of Ob/Gyn and Reproductive Sciences, Center for Reproductive Sciences, Eli and Edythe Broad Center for Regeneration Medicine & Stem Cell Research, San Francisco, CA, USA.

Abstract

A critical step in the development of gametes is the migration of their primordial germ cell (PGC) precursors to the forming gonads. Although the location and mode of PGC specification differs between organisms, the formation of a committed germline before organogenesis creates a need for migration through the growing embryo in order to reach the site of gonadogenesis. Failure of PGC migration can, in many cases, compromise fertility or conversely lead to the formation of teratomas in sites outside of the gonad. Here we review the mechanism of migration employed by PGCs and compare the timing and routes across several model organisms. We summarize recent work on the role of the Wnt signaling pathway in cell migration and the lineage specific function in PGCs, mainly through the ligand Wnt5a and its receptor Ror2.

Keywords: cell migration, primordial germ cell, Ror2, Wnt signaling, Wnt5a.

Introduction

Although the egg and sperm are not used until adulthood, their precursors, the primordial germ cells (PGCs), are among the first lineages established in development (Laird *et al*., 2008). Due to their critical role in preserving genomic integrity and transmitting genetic information to the next generation, the development of PGCs from their initial specification to final maturation has been studied in multiple species to identify the processes and pathways that safeguard their reproductive function. In all model organisms studied thus far, the PGCs are specified far from their ultimate residence in the gonads; thus, they must initiate and complete a lengthy migration through the developing embryo from their point of origin to reach the gonadal primordia (Richardson and Lehmann, 2010). An adequate supply of gametes depends upon the successful migration of PGCs during embryogenesis, with the failure to complete this journey resulting in infertility and an increased risk of teratoma development. Therefore, precise control of PGC movement, interactions with a diversity of cellular environments, and survival en route to the gonad is essential for ensuring the reproductive success of an organism. Here we review cell migration as it relates to the germline. We examine and compare the developmental timing and cellular routes of PGC migration in multiple species, as well as explore the molecular signaling that controls this process, with a particular emphasis on the Wnt pathway.

Cell migration in development

Cell migration is vital for development and survival and has been observed in the simplest to most complex multicellular organisms. A number of events have been identified in the coordination of cell motility including asymmetric polarization, protrusion formation at the leading edge, cell adhesion, and translocation or retraction of the lagging edge (Izzard and Lochner, 1980; Chen, 1981; Coates *et al.,* 1992; Lauffenburger and Horwitz, 1996; Chen *et al.,* 2003). There are many types of motile cells which each deploy unique and context-dependent signaling pathways to mediate these fundamental processes; however, nearly all forms of migration require reorganization of the actin cytoskeleton and integrin binding to the extracellular matrix (ECM). External cues such as extracellular substrate stiffness, combinations of secreted factors, and two versus three-dimensional surroundings are also important in regulating cell migration (Pelham and Wang, 1997; Lo *et al.,* 2000; Lämmermann *et al.,* 2008; Hynes, 2009; Pathak and Kumar, 2012; Charras and Sahai, 2014).

The two major categories of cell movement are collective cell migration and single cell migration. The primary task of each mode of migration differs substantially - collective migration drives the assembly, form, and regeneration of complex tissues and organs and single cell migration allows cells to move between locations, to integrate into tissues or perform effector functions (Friedl and Wolf, 2010).

Collective cell migration

The most common form of cell movement during development is collective cell migration, a process defined by the coordination and cooperation of neighboring cells to move together as a group. Collective cell migration has been observed in epithelial tissues such as the lateral line primordium in zebrafish or mammalian vasculature (Metcalfe, 1985; Rousseau *et al.,* 1997) as well as mesenchymal cohorts like those of the neural crest in *Xenopus* and mouse (Serbedzija *et al.,* 1990; Collazo *et al.,* 1993). Despite the use of different molecular and genetic pathways depending on cell type and species, the underlying principles of collective cell migration are conserved. The defining feature of this form of cell movement is the maintenance of stable cell-cell contacts between neighboring motile cells (Hegerfeldt *et al.,* 2002; Ulrich *et al.,* 2005; Llense and Martín-Blanco, 2008). Through these interactions, cells of the collective are able to

[1]Corresponding author: diana.laird@ucsf.edu
Phone: (415) 476-5471
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specialize leaders and followers, propagate directional cues, and maintain polarity within the group (Scarpa and Mayor, 2016). Cells that move together have increased efficiency of migration as compared to cells that move individually, because they can collectively buffer heterogeneity in cell responsiveness to maintain direction and substantially remodeling the extracellular environment to form a clear route for the follower population (Vitorino and Meyer, 2008; Gjorevski *et al.,* 2015). To more easily define and manipulate the mechanisms of collective cell migration, *in vitro* models of wound healing, matrix invasion, and planar cell polarity have been developed using fibroblasts, migratory cell lines, or primary cells (Friedl and Gilmour, 2009).

Single cell migration

While many classical studies of cell movement used models of collective cell migration, there are several examples of cells that migrate singly in development, homeostasis, and disease. In the embryo, hematopoietic stem cells, immature neurons, and PGCs migrate as single cells from their point of specification to their final location (Chiquoine, 1954; Berry and Rogers, 1965; Johnson and Moore, 1975; Forrester and Garriga, 1997). In adult homeostasis, leukocytes survey the body for wounding and infection by migrating through various tissues and the vasculature (de Bruyn, 1946; Miller *et al.,* 2003; Weninger *et al.,* 2014). In cancer, metastatic cells move out of the primary tumor by hijacking components of the migratory network to colonize new sites around the body (Clark and Vignjevic, 2015).

The movement of single cells is similar in general principle to movement by groups of cells – they must polarize, sense chemotactic signals, and form adhesions with their extracellular surroundings; however, in contrast to collect cell migration, each individually migrating cell must be capable of all functions. This often results in higher cell velocities, because there is no negotiation with neighboring cells, but lower overall efficiency of directed migration, since single cells are more susceptible to subtle changes in extracellular signaling and thus more likely to wander. *In vitro* culture assays are currently being used to study how individual cells interact with the extracellular compartment and simultaneously control the multitude of processes that allow for motility.

Primordial germ cell migration

Conservation of primordial germ cell migration

In many species, the PGCs are among the earliest cell lineages specified in the embryo, often set aside prior to gastrulation and far from their ultimate residence in the gonads (Chiquoine, 1954; Ephrussi and Lehmann, 1992; Yoon *et al.,* 1997). Thus, PGCs must undergo a lengthy and active migration through the developing embryo to reach the somatic cells of the developing gonads (Fig. 1; Richardson and Lehmann, 2010). Fertility in the adult relies upon the successful migration of the germline; failure to complete this process results in a loss of functional germ cells and increased risk for the development of germ cell tumors (Mintz and Russell, 1957; McCoshen and McCallion, 1975; Chaganti *et al*., 1994). Through the study of multiple model organisms, it has been shown that the overall process of PGC migration - interaction with multiple tissue types, receptivity to and sensing of chemoattractant and repellant cues - is highly conserved, and several key genes and signaling pathways have been identified (Richardson and Lehmann, 2010).

In *Drosophila melanogaster* (fruit fly), the process of PGC migration is completed within 4 h (Sonnenblick, 1941; Starz-Gaiano and Lehmann, 2001). During gastrulation, the PGCs are passively carried into the midgut pocket of the developing embryo (Jaglarz and Howard, 1995). Shortly thereafter, the PGCs begin to express Tre1, which polarizes the cells and initiates their individual dispersal through the epithelial layer midgut endoderm (Fig. 1; Kunwar *et al.,* 2008). Once outside the midgut, *Drosophila* PGCs migrate dorsally then anteriorly along the midgut, incorporate into the posterior mesoderm, and bifurcate laterally to join somatic gonadal precursor cells (SGPCs) and form the gonads (Sonnenblick, 1941; Warrior, 1994). This migratory route is defined by the precise expression of Wunens (Wun, Wun2) in the neighboring somatic tissues, such as the midgut, which act as repellant signals to guide PGCs toward the SGPCs and restrict movement into ectopic locations (Zhang *et al.,* 1996, 1997; Starz-Gaiano *et al.,* 2001). Later-stage migration to the lateral mesoderm and eventual association with the SGPCs is directed by the expression of Hmgcr, an enzyme important in cholesterol and isoprenoid synthesis, and Mdr49, an export protein, in the soma; however, the identity of the chemoattractant produced and secreted by these proteins has yet to be determined (Van Doren *et al.,* 1998; Ricardo and Lehmann, 2009). Motility in *Drosophila* PGCs is mediated by the downregulation of DE-cadherin and formation of actin-rich leading and lagging edges to coordinate protrusion, adhesion, and retraction of the cells (Jaglarz and Howard, 1995; Kunwar *et al.,* 2008).

Another well-studied model for PGC migration is *Danio rerio* (zebrafish). Over the course of 18 h, zebrafish PGCs embark on a complex journey through a series of intermediate targets on their way to the final location of the gonad (Weidinger *et al.,* 1999, 2002). Following their specification in four random locations throughout the embryo (Yoon *et al*., 1997; reviewed in Raz, 2003), a feature unique to zebrafish development, the PGCs migrate to the dorsal side of the embryo, are excluded from the midline, align with the anterior and lateral mesoderm, and coalesce into two lateral clusters which are moved posteriorly to their final position at the site of gonad formation (Weidinger *et al.,* 1999). Coordination of this movement is mediated primarily by the chemoattractant SDF1 (also known as CXCL12), which has a spatially and temporally dynamic expression that is tightly regulated in somatic tissues that comprise the migratory route (Doitsidou *et al.,*

2002; Knaut *et al.,* 2003). Additionally, the isoprenoid arm of Hmgcr pathway is important in zebrafish PGC migration, although its exact mechanism of action has not been elucidated (Thorpe *et al*., 2004). Unlike germ cells in other organisms which polymerize actin and modify the cytoskeleton to form protrusions at the leading edge, PGCs in the zebrafish embryo employ membrane blebbing and cytoplasmic flow as a core means of their motility (Blaser *et al.,* 2006). The PGCs switch between periods of active and directional movement termed "running," and "tumbling," or pauses in movement characterized by environment-sensing via multiple membrane blebs and reorientation toward the chemotactic signal guiding their migration to the gonads (Reichman-Fried *et al.,* 2004). This style of migration is considered to be a strategy for PGCs to move forward via cytoplasmic translocation when adhesions with the surrounding microenvironment are overly stable or cannot be altered quickly enough to permit more traditional movement (Paluch and Raz, 2013).

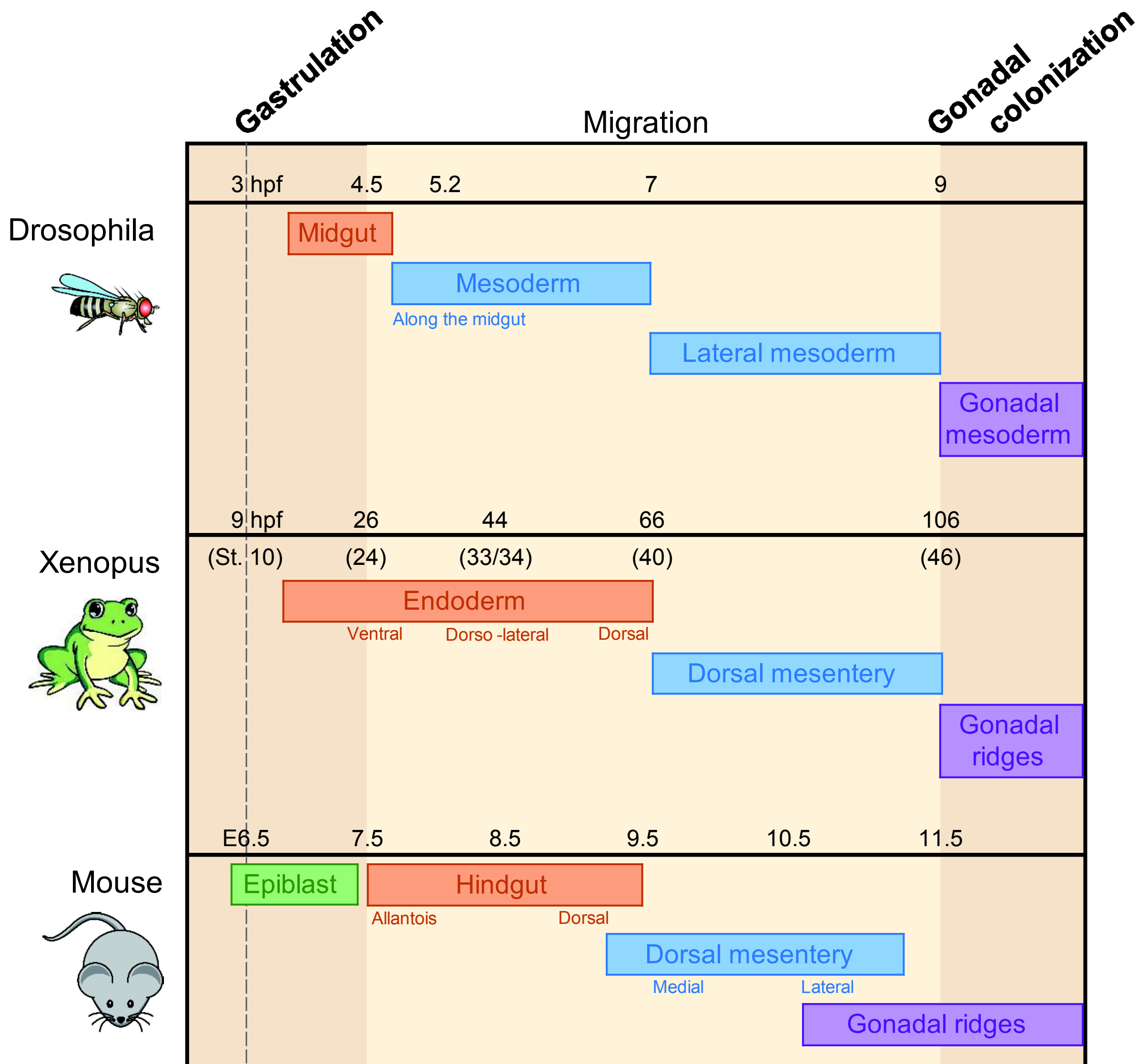

Figure 1. Conservation of PGC migration between multiple species. Following gastrulation (dashed line), PGCs in Drosophila, Xenopus, and mouse undergo lengthy migrations through endodermal sheets (orange) and mesodermal tissues (blue) to reach the developing gonads (purple). Time scales of the migratory period are noted for each species; hpf = hours post-fertilization, E = embryonic day. Annotations underneath each bar represent specific locations and patterns of movement through that region.

Other model organisms in which PGC migration has been described, but not yet rigorously studied, include *Gallus gallus* (chicken) and *Xenopus laevis* (frog). In the chicken, PGCs are moved passively by gastrulation to the germinal crescent, the region of extraembryonic tissue anterior to the head. From the germinal crescent they concentrate in the sinus terminalis, enter the embryonic vasculature via the vitelline veins, and utilize the circulatory system to reach the developing gonadal soma in the intermediate mesoderm (Nakamura *et al*., 2007; de Melo Bernardo *et al*., 2012). This process occurs over approximately 1.5 days, from stages 9 to 17 of embryonic development. The presence of protrusions on chick PGCs in the

mesoderm (Nakamura *et al.,* 2007) and the similarities in movement with leukocytes during inflammation (reviewed in Weninger *et al.,* 2014) suggest that migration through the endothelium and within the mesoderm is an active process. In situ hybridization revealed high levels of SDF1 expression in the mesodermal tissues of later chick PGC migration and concordant expression of CXCR4 on the germ cells (Stebler *et al.,* 2004). Ectopic expression of SDF1 in the chick embryo re-routed many PGCs to new locations, suggesting that this chemokine guides exit of the germ cells from the vasculature and subsequent interstitial tissue movement toward the gonads (Stebler *et al.,* 2004). Other guidance cues, mechanisms of motility, and signaling pathways important for PGC migration in the chicken embryo remain unknown.

In the frog, PGC migration spans two days of development, from stages 24 to 46 (Kamimura *et al.,* 1976; Nishiumi *et al.,* 2005). Following localization to the ventral endoderm during gastrulation, PGCs dissociate from their original cluster and begin actively migrating dorsally, laterally, and anteriorly through the endodermal mass (Fig. 1). Upon reaching the most dorsal region of the endoderm, they exit into the dorsal mesentery and reaggregate in the genital ridges. The chemotactic signals that direct the movement of frog PGCs have not yet been identified; however, recent work has begun to elucidate the role of adhesive properties, ECM molecules, and internal cytoskeletal dynamics in regulating the modes of PGC motility in the developing frog embryo (Nishiumi *et al.,* 2005; Dzementsei *et al.,* 2013; Terayama *et al.,* 2013).

PGC migration in the mouse

Due to the limiting number of PGCs in the early stages of development, much less is known about the mechanisms that regulate germ cell migration in mammals as compared to fly or zebrafish. After specification in the epiblast, mouse PGCs begin their migration on embryonic day 7.5 (E7.5) by actively entering the primitive endoderm, which will differentiate into the hindgut (Chiquoine, 1954; Clark and Eddy, 1975; Anderson *et al.,* 2000). Over the course of several days, they integrate into and travel within the developing hindgut epithelium before crossing the basement membrane at E9.5 and invading into surrounding mesentery (Fig. 1). The PGCs then move dorsally through the elongating dorsal mesentery of the midline before bifurcating laterally and ultimately colonizing the emerging gonads from E10.5-11.5 (Molyneaux *et al.,* 2001; Richardson and Lehmann, 2010). Once in the gonad, mouse PGCs, like other species, lose their migratory potential and initiate sex differentiation pathways (Donovan *et al.,* 1986). Similar to the migration in all model organisms that have been studied, mammalian PGCs interact with both epithelial and mesenchymal tissues and undergo periods of active and passive movement during the migratory period.

Several secreted factors and their corresponding receptors have been identified in the promotion of motility and directional guidance of migratory mouse PGCs. The best characterized ligand-receptor pair in PGC migration is KitL (also known as Steel factor or Stem cell factor) and cKit. Early studies of mice carrying mutations in the cKit locus (also known as W) or KitL locus (also known as Steel) noted a dramatic reduction in population size of the germline as well as a delay in the migration of PGCs that resulted in failure to efficiently colonize the gonadal ridges (Mintz and Russell, 1957; McCoshen and McCallion, 1975; Buehr *et al.,* 1993; Runyan *et al.,* 2006). Careful characterization of KitL, which has both membrane-bound and secreted forms (Flanagan *et al.,* 1991; Huang *et al.,* 1992; Miyazawa *et al.,* 1995), revealed its expression by somatic cells of the embryonic mesoderm and gonadal ridges to be spatially and temporally dynamic along the migratory route, creating a wave of expression coined the “traveling niche” (Gu *et al.,* 2009). While secreted KitL is present and capable of facilitating PGC movement, this wave is generated by the membrane-bound form of KitL in order to produce a localized high concentration of ligand needed to sustain and guide PGC motility (Gu *et al.,* 2011). Addition of exogenous secreted KITL in *ex vivo* cell culture induces polarization and protrusion formation in migratory PGCs and drives the movement of post-migratory PGCs in embryo slice culture (Farini *et al.,* 2007; Laird *et al.,* 2011; Gu *et al.,* 2011).

Conserved chemotactic pathways have also been identified in the mouse, although they are less well-studied. The SDF1-CXCR4 pathway has been established as a mediator of PGC migration (Ara *et al.,* 2003; Molyneaux *et al.,* 2003). Akin to its role in zebrafish, SDF1 is thought to function primarily as a chemoattractant in the later stages of migration to the gonadal ridges, as initiation of PGC migration and entry into the hindgut is unaffected by genetic loss of the ligand or receptor. In another parallel to fly and fish, the Hmgcr pathway plays a role in mouse PGC migration; however, its function is more complex and utilizes both the cholesterol and isoprenoid arms of the pathway (Ding *et al.,* 2008).

Adhesion molecules and ECM proteins are also critical in ensuring efficient migration of PGCs to the gonads. Expression of several common ECM components has been found along the migratory route, notably collagen IV, fibronectin, and laminin in the mesentery and laminin in the basement membranes of the mouse hindgut and coelomic epithelia (De Felici and Dolci, 1989; Ffrench-Constant *et al.,* 1991; García-Castro *et al.,* 1997). Cell culture experiments highlighted the ability of PGCs to bind each of these molecules to varying degrees depending on substrate and age of the germ cells. Loss of a major ECM binding protein, β1-integrin, results in a migratory delay in PGCs, with a majority of mutant germ cells remaining outside the gonads at E11.5 (Anderson *et al.,* 1999). Several α-integrins are also known to be expressed by PGCs, but their removal does not impair migration, suggesting a redundancy in the ability to bind ECM which may correspond to the overlapping expression patterns of different ECM proteins. The one area of ECM interaction that has been understudied is the

identification of degradation molecules that allow for PGC invasion through the basement membrane of the hindgut as well as movement within the different tissues along the migratory route, and thus, remains an open question.

In addition to the ECM, cell-cell interactions are likely important in the regulation of PGC migration. Similar to their counterparts in the fly, frog, and zebrafish (Blaser *et al.,* 2005; Kunwar *et al.,* 2008; Baronsky, *et al.,* 2016), mouse PGCs express E-cadherin (Bendel-Stenzel *et al.,* 2000; Di Carlo and De Felici, 2000), a classic mediator of cell-cell contact and stable adhesion, especially within epithelial tissues. However, distinct from these other organisms which downregulate E-cadherin prior to migration, PGCs in the mouse maintain and utilize its expression throughout their migration from the hindgut to the gonads (Bendel-Stenzel *et al.,* 2000; Di Carlo and De Felici, 2000). The presence of E-cadherin on PGCs during this time is puzzling given its absence in tissues beyond the hindgut; thus, it was proposed that E-cadherin is used to form a network of migrating PGCs by maintaining cell-cell contact through very long and thin protrusions between germ cells (Gomperts *et al.,* 1994). However, time lapse imaging of embryo slice cultures did not find evidence of sustained contact between PGCs as suggested by images from histological sections (Molyneaux *et al.,* 2001), so the function of E-cadherin during migration remains a mystery.

Other key cellular processes during the migratory period in mouse PGC development

Simultaneous with their migration, PGCs are also coordinating several other cell processes important for their development. Distinct from other model organisms (Su *et al.,* 1998; Richardson and Lehmann, 2010; de Melo Bernardo *et al*., 2012), mammalian PGCs are actively proliferating during their migration, increasing in population size from approximately 45 cells at E7.5 to ~200 at E9.5 (Saitou *et al*., 2002; McLaren, 2003; Seki *et al*., 2007), ~2500 at E11.5 (Laird *et al.,* 2011), and peaking around 25,000 at E13.5 (Tam and Snow, 1981). The KitL-cKit pathway is one regulator of germ cell proliferation, with several genetic mutants for either ligand or receptor unable to expand their number of PGCs after specification, resulting in fertility defects (Mintz and Russell, 1957; McCoshen and McCallion, 1975; Buehr *et al.,* 1993). Adding to its functions as a mitogen and chemoattractant, KitL has also been shown to play a role in PGC survival (Runyan *et al.,* 2006).

In vitro cultures of migratory PGCs have confirmed the proliferation and survival functions of KitL (Godin *et al.,* 1991; Dolci *et al.,* 1991; Matsui *et al.,* 1991) and identified a number of other factors important for these processes including LIF (Matsui *et al.,* 1991), FGFs (Resnick *et al.,* 1992; Kawase *et al.,* 2004), SDF1 (Molyneaux *et al.,* 2003), forskolin (De Felici *et al.,* 1993), retinoic acid (Koshimizu *et al.,* 1995), and TGFβ1 (Godin and Wylie, 1991). Refinement of germ cell culture conditions has led to a reduction in the factors necessary to maintain the survival of PGCs, increasing the utility of this system in identifying and testing the function of new factors in all facets of germ cell development (Laird *et al.,* 2011).

In addition to proliferation, PGCs are also undergoing epigenetic reprogramming via several different mechanisms during their migration to the gonadal ridges (Seki *et al.,* 2005). This reprogramming is thought to be critical for PGC development and function, by preventing differentiation down somatic lineages, maintaining the expression of pluriopotency genes, and erasing imprinted marks to allow for their reset during the process of gametogenesis (Ancelin *et al.,* 2006; De Felici, 2011; Seisenberger *et al.,* 2012; Hackett *et al.,* 2013). Shortly after specification, levels of DNA methylation and the types of histone marks found in PGCs are indistinguishable from their somatic neighbors (Seki *et al.,* 2005, 2007). However, as the PGCs proceed through migration, they begin to demethylate cytosines in CpG islands across their genome, resulting in distinctly different epigenetic patterning at E11.5 than that seen at E8.0 or in the somatic cells (Kafri *et al.,* 1992; Seki *et al.,* 2005; Seisenberger *et al.,* 2012; Hackett *et al.,* 2013). This process of demethylation is not indiscriminate, as several post-migratory PGC genes, including Vasa, Scp3, and Dazl, imprinted genes, and retrotransposons remain highly methylated until after gonadal colonization (Maatouk *et al.,* 2006; Hackett *et al.,* 2012; Seisenberger *et al.,* 2012). Additionally, the histone modifications that occur during migration, including erasure of H3K9me2 and addition of H3K27me3 and H4/H2AR3me2, appear to move the genome toward greater transcriptional plasticity while preventing inappropriate differentiation (Seki *et al.,* 2005; Ancelin *et al.,* 2006). It remains unclear if this phase of epigenetic reprogramming is linked to PGC migration and movement through different somatic microenvironments or intrinsically regulated by developmental timing, and insufficient numbers of PGCs during this period have prevented the use of experimental techniques typical in this line of research. However, it may be possible to parse the question of intrinsic timing versus somatic control by examining the epigenetic status of PGCs in migration mutants in which germ cells are found in the wrong place at the wrong time.

Noncanonical Wnt signaling in cell migration

Canonical versus noncanonical Wnt signaling

Wnt signaling is one of the most commonly utilized and well-studied pathways in development. The Wnt family of genes was originally discovered in a screen for proto-oncogenes in mammary cancer (Nusse and Varmus, 1982). Characterization of this original Wnt, Wnt1 (formerly known as Int1) identified the transcription factor β-catenin as a key effector of its signaling pathway (McMahon and Moon, 1989; McCrea *et al.,* 1993; Young *et al.,* 1998; Mizushima *et al.,* 2002). The ensuing identification of more Wnt family members

and their downstream pathways led to the classification of two distinct arms of the Wnt pathway: canonical, β-catenin-mediated signaling and noncanonical, non-β-catenin-mediated signaling (Niehrs, 2012).

Canonical Wnt signaling is defined by its activity in the "on" or "off state." In the "off state" of signaling, cytoplasmic β-catenin is bound by the Axin-GSK3β-APC-CK1ε complex, phosphorylated, and degraded (Hart *et al.,* 1998; Logan and Nusse, 2004). However, engagement of Frizzled and LRP receptors by Wnt ligands turns "on" the pathway, causing dissociation of this degradation complex. This allows β-catenin to accumulate in the cytoplasm and translocate to the nucleus, where it binds transcription factors in the LEF/TCF family and induces gene expression (Behrens *et al.,* 1996; Molenaar *et al.,* 1996; Niehrs, 2012). This arm of the pathway regulates cell specification and fate decisions, organogenesis, and proliferation (Gat *et al.,* 1998; Korinek *et al.,* 1998; Wang *et al.,* 2012).

Noncanonical Wnt signaling is an all-encompassing term for Wnt pathways that do not work through β-catenin, of which there are many. In these pathways, Wnts commonly engage Frizzled and non-LRP receptors, such as Ror or Ryk family members (Hikasa *et al.,* 2002; Oishi *et al.,* 2003; Wouda *et al.,* 2008), and activate a variety of downstream signaling pathways (Nishita *et al.,* 2010a; Niehrs, 2012). Regulation of planar cell polarity is the best studied function of noncanonical Wnt signaling; however, these pathways can also regulate multiple mechanisms of cell migration and invasion as well as inhibit the canonical Wnt pathway (Mikels and Nusse, 2006; Nishita *et al.,* 2006; Enomoto *et al.,* 2009; Mikels *et al.,* 2009).

While early studies attempted to classify Wnts and their binding partners as canonical or noncanonical, recent work suggests that the context of receptors and ligands determines the predominant downstream signaling pathway in an individual cell (van Amerongen and Nusse, 2009; van Amerongen *et al.,* 2012). This promiscuity of Wnt function can thus explain their broad expression throughout development and ability to regulate many cell types and processes at the same time.

Wnt signaling in germ cell development

Several Wnt ligands have been implicated in PGC development in the mouse: Wnt3 and Wnt3a in specification (Ohinata *et al*., 2009; Bialecka *et al*., 2012; Aramaki *et al*., 2013; Tanaka *et al*., 2013), Wnt5a, and its receptor Ror2, in migration (Laird *et al*., 2011; Chawengsaksophak *et al*., 2012), and Wnt4 in female sex-differentiation (Vainio *et al*., 1999; Chassot *et al*., 2012). Both the specification and sex-differentiation of PGCs utilize the *β-catenin*-dependent, canonical arm of the Wnt pathway (Chassot *et al*., 2008, 2011; Aramaki *et al*., 2013), while migration is regulated by the noncanonical Wnt pathway (Laird *et al.,* 2011; Chawengsaksophak *et al.,* 2012). Similar regulation of PGC migration by Wnts has been shown in Drosophila via WntD and is likely mediated through signaling independent of the canonical pathway (McElwain *et al.,* 2011). Overactivation of the canonical Wnt pathway in post-migratory mouse PGCs has been shown to slow the cell cycle rate and reduce the size of the germ cell pool (Kimura *et al.,* 2006).

The noncanonical Wnt receptor Ror2

As previously mentioned, noncanonical Wnt signaling is defined by the binding of Wnt to Frizzled and non-LRP receptors (Niehrs, 2012). One such receptor is Ror2, a tyrosine kinase-like receptor that is highly conserved and plays a critical role in the development of many organs and tissues (Minami *et al.,* 2010). Ror-family members are found in invertebrate and vertebrate species ranging from *C. elegans* to humans (Forrester and Garriga 1997; Forrester *et al.,* 1999; DeChiara *et al.,* 2000; Oldridge *et al.,* 2000; Takeuchi *et al.,* 2000; Hikasa *et al.,* 2002). The mammalian Ror2 protein consists of several domains including an extracellular cysteine-rich domain that is similar to those found in Frizzled proteins, an extracellular Kringle domain with unknown function in Ror2, an intracellular tyrosine kinase domain the phosphorylation of which is not always required for Ror2 signaling, and an intracellular proline-rich domain that binds cytoskeletal modifiers (Patthy *et al.,* 1984; Oishi *et al.,* 2003; Nishita *et al.,* 2006; Liu *et al.,* 2008). Loss of Ror2 in the mouse results in numerous developmental abnormalities including a shortened body axis, defects in limb and genital outgrowth, cleft palate, and respiratory and cardiac dysfunction that result in death shortly after birth (DeChiara *et al.,* 2000; Takeuchi *et al.,* 2000; Oishi *et al.,* 2003). Similar defects are found in mouse mutants of Wnt5a, a classically described noncanonical Wnt and the most characterized ligand for Ror2 (Yamaguchi *et al.,* 1999). Mutations in the human Ror2 locus are associated with Robinow syndrome, a disorder characterized by dwarfism, craniofacial defects, and genital hypoplasia (Afzal *et al.,* 2000; van Bokhoven *et al.,* 2000), and brachydactyly type B, disorder in the growth of distal phalanges and nails (Oldridge *et al.,* 2000; Schwabe *et al.,* 2004).

Wnt5a and Ror2 have been shown to regulate many downstream signaling pathways. Work in cell lines identified a role for Wnt5a suppression of canonical Wnt signaling via Ror2 (Mikels and Nusse, 2006); this interaction between the Wnt pathways was confirmed in *in vivo* studies where loss of Ror2 lead to an increase in canonical Wnt activity (Mikels *et al.,* 2009). Wnt5a-Ror2 have also been linked to the planar cell polarity (PCP) pathway through several *in vivo* studies. In the developing limb bud, Wnt5a is expressed in a gradient that corresponds with a gradient of phosphorylated Vangl2, a core protein involved in PCP (Gao *et al.,* 2011). This phosphorylation is mediated by Ror2. In the developing cochlea, Wnt5a regulates the polarized distribution of Vangl2 and maintains the polarity and organization of hair cells (Qian *et al.,* 2007). Both groups also showed that Wnt5a;Vangl2 or Ror2;Vangl2 double-mutant embryos failed to close the neural tube, a defect common to mutants of key

PCP genes, further implicating Wnt5a-Ror2 in this pathway (Qian *et al.,* 2007; Gao *et al.,* 2011).

Wnt5a-Ror2 in cell migration

In addition to regulation of planar cell polarity and suppression of canonical Wnt signaling, a majority of noncanonical Wnt signaling pathways have been linked to various forms and mechanisms of collective and single cell migration (Nishita *et al.,* 2010). Work in numerous cell lines has identified Wnt5a-Ror2 as upstream regulators of cell migration through protrusion formation or calcium signaling and cell invasion via ECM degradation (Slusarski *et al*., 1997; Nishita *et al.,* 2006; Enomoto *et al*., 2009). Downstream effectors of Wnt5a and Ror2 in these pathways include Dishevelled, JNK, c-Src, and Filamin A (Nishita *et al.,* 2006; Akbarzadeh *et al.,* 2008; Nomachi *et al.,* 2008; O'Connell *et al.,* 2009; Nishita *et al.,* 2010b).

In vivo studies have identified a role for Ror2 in directing neuronal migration in *C. elegans* and Wnt5a-Ror2 in regulating convergent extension during gastrulation in *X. laevis* (Forrester and Garriga 1997; Forrester *et al.,* 1999; Hikasa *et al.,* 2002). Recent publications have further implicated Wnt5a-Ror2 in various processes of mammalian cell migration including mediation of chemotactic responses, convergent extension, and cell invasiveness in the palate, gut, and osteosarcoma lines, respectively (He *et al.,* 2008; Enomoto *et al.,* 2009; Yamada *et al.,* 2010). Ror2 as a biomarker and therapeutic target for metastatic cancers is also an active area of research (Morioka *et al.,* 2009; Wright *et al.,* 2009; Debebe and Rathmell, 2015).

Wnt5a-Ror2 in PGC migration

Our lab identified a recessive allele of Ror2 in a genome-wide ENU mutagenesis screen for genes involved in germ cell development, establishing the first connection between Ror2 and PGCs (Laird *et al.,* 2011). In $Ror2^{Y324C/Y324C}$ ($Ror2^{Y324C}$) mutants, PGCs accumulated in ectopic and extra-gonadal locations and colonized the gonadal ridges with poor efficiency. Examination of cell shape (by elongation index, EI) and angle of cell axes in these Ror2-mutant PGCs *ex vivo* demonstrated an inability to elongate and orient appropriately in response to KitL, suggesting a cellular mechanism for their migratory defect. Measurements of E9.5 $Ror2^{Y324C}$ PGCs *in vivo* revealed elongation defects similar to those seen *in vitro,* with mutant PGCs adopting the rounded morphology of post-migratory WT PGCs that lost their motility after arrival in the gonads at E11.5. We recently found that the impaired migratory capacity of $Ror2^{Y324C}$ PGCs contributes to a failure to colonize the anterior tip of the gonadal ridges, and that this aberrant distribution of PGCs persists in the fetal ovary and leads to a delay in the initiation of meiosis across the population (Arora *et al*., 2016).

Together, these data show that Ror2-mutant PGCs are less competent to migrate during development, although the precise mechanism for this defect is unknown.

Wnt5a is similarly important in PGC migration and development, with mutants showing a more profound loss of germ cells colonizing the gonadal ridges than $Ror2^{Y324C}$ and disruption of testicular development in the male (Chawengsaksophak *et al.,* 2012). Broad expression of Wnt5a in somatic tissues along the migratory route suggests that this ligand does not provide guidance cues to PGCs (Laird *et al.,* 2011). This was confirmed by experiments in which Wnt5a-coated beads did not divert PGCs from their migratory route (Laird *et al.,* 2011). Interestingly, Wnt5a-coated beads implanted in the developing mouse palate induced attraction and directional migration of mesenchymal cells toward the beads (He *et al.,* 2008); this Wnt5a-mediated migration was dependent on Ror2. Together, these studies raise the possibility that Wnt5a has chemotactic properties in some migratory contexts but not others.

In addition to facilitating PGC migration, recent studies suggest that the Wnt5a-Ror2 signaling axis plays an additional role in proliferation. Closer examination of Ror2 mutant PGCs revealed an elevation of cell cycle genes and overproliferation specifically in the hindgut, where WNT5A expression is highest. It was shown genetically that the β-catenin-dependent canonical pathway promotes proliferation and is repressed by Wnt5a via Ror2 in PGCs (Cantú *et al.,* 2016). The implication is that Wnt5a and Ror2 function to balance movement with proliferation by toggling between different arms of the Wnt signaling pathway.

Outstanding questions

The studies on Wnt5a and Ror2 summarized above raise new questions, namely which aspects of cell migration are autonomous and which are regulated by the surrounding somatic environment. *Ex vivo* culture data suggested a PGC-autonomous role for Ror2 in regulating migration, but broad expression of the receptor and its ligand in somatic tissues of the embryo (Yamada *et al.,* 2010; Laird *et al.,* 2011) raise the possibility that Ror2 function in cells of the migratory niche might influence PGC motility and development. Since the precise signaling pathways by which the Wnt5a-Ror2 pathway regulates migratory PGCs were not previously identified, questions remain about how Wnt5a regulates PGC migration without providing directional cues and whether the canonical Wnt pathway is relevant to this period of germ cell development. The evolutionary conservation of Wnt5a-Ror2 signaling in PGC development remains to be seen through the study of other model organisms. Finally, the use of conditional alleles will bypass the lethality of loss of Wnt5a-Ror2 signaling and permit interrogation of the consequence of reduced motility of PGCs on fertility.

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